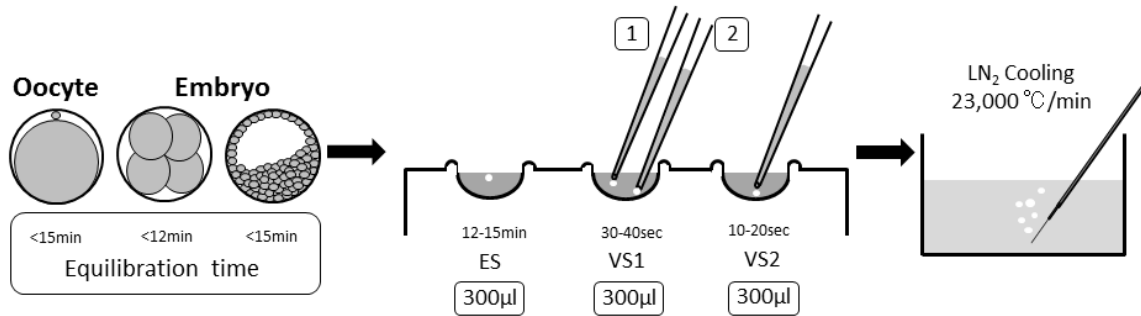


## VITRIFICATION KIT (101)



### Contents of the Kit

- Equilibration Solution (ES): 1 vial of 1.0ml.
- Vitrification Solution (VS): 2 vials of 1.0ml.
- 4 Cryotecs: 1 cryotec can contain more than one oocyte/embryo (recommend 3-4 maximum for oocytes and 4-cells embryos, and one for blastocyst for single embryo transfer).
- 3 Vitri Plates with 3 wells each.

### Instructions:

#### Preparation

- The whole process should be performed under room temperature (25-27°C).
- Fill a nitrogen container.
- Compare the thickness of the zona pellucida with the perivitelline space, and take note for oocyte.
- Important: Use a Pasteur pipet with the right diameter for oocyte, embryo (140-150 µm) and Blastocyst (160-200 µm).

#### Equilibration of oocytes and embryos

1. Fill the Vitri Plate with 300 µl of ES in the 1<sup>o</sup> well, and 300 µl of VS in the 2<sup>o</sup> and 3<sup>o</sup> well.
2. Put the oocyte/embryo on the surface of ES in the 1<sup>o</sup> well.
3. The oocyte/embryo will sink and begin to shrink, and gradually returns to the original size (maximum 15 min for oocyte and blastocysts, and 12 min for other stages of embryos).

#### Vitrification

**Attention:** The following steps must be made in no less than 25 sec and a maximum time of 90 sec.

4. Transfer the oocyte/embryo to the half depth of the 2<sup>o</sup> well with VS. (Not with minimum volume of ES at the first step) The oocyte/embryo immediately floats to the surface of VS while washed.
5. After washing the inside wall of the pipette with fresh VS media, take only the oocyte/embryo and transfer it to the bottom of the well. Wait until the oocyte/embryo floating stops in VS.
6. Transfer the oocyte/embryo to the middle depth of the 3<sup>o</sup> well with VS, and mix the media by pipette around for 5 times.
7. Take only the oocyte/embryo at the top end in the pipette, and put it on the end of the cryotec seat with minimum volume of VS.
8. Immediately submerge the Cryotech into liquid nitrogen.
9. Place the cap, and store it in a nitrogen tank.

Please stay Cryotec in liquid nitrogen at all times.

[www.cryotechlab.com](http://www.cryotechlab.com)

### Quality Control Tests:

This Lot N<sup>o</sup> JIHA0115 (All Solutions)

Successfully passed the following controls:

- Sterility : Sterility test .
- Endotoxin by ES methodology (Each component).
- Efficiency: survival of 50/50 Mouse embryos and Porcine oocytes.

### Storage and stability

Solutions and kits can be transported under the room temperature, and then must be kept in the fridge at 2-8°C until the expiration date.

### Composition

- Modified HEPES Buffered MEM
- Hydroxy Propyl Cellulose
- Ethylene Glycol
- Dimethyl Sulfoxide
- Endotoxin free Trehalose

### References

- Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: The CryoTop method. *Theriogenology* 67, 73-80, 2007.
- Cobo A, Kuwayama M. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. *Fertil Steril.* J89(6): 1657-64, 2007.
- Antinori M, Licata E, Dani G, Cerusico F, Versaci C, Antinori S. Cryotop vitrification of human oocytes results in high survival rate and healthy deliveries. *Reproductive BioMedicine Online* 14, 5-667, 2007.
- Vajta G, Kuwayama M. Improving cryopreservation systems. *Theriogenology* 65(1), 236-44, 2006.
- Kuwayama M. Highly efficient vitrification method for cryopreservation of human oocytes. *Reproductive BioMedicine Online* 11:300-308, 2005.
- Ushijima J, Kuwayama M. High survival rate of bovine oocytes matured in vitro following vitrification. *J Reprod Dev.* 50:685-96, 2004.
- Fukui Y, Kuwayama M. Effect of cryodevice type and donor's sexual maturity on vitrification of minke whale oocytes at germinal vesicle stage. *Zygote* 12, 333-338, 2004.
- Hochi S, Kuwayama M. Improved Survival of Vitrified in vivo-derived porcine embryos. *J. Reprod. Develop.* 50, 481-486, 2004.
- Esaki R, Kuwayama M. Cryopreservation of porcine embryos derived from in vitro-matured oocytes. *Biology of Reproduction.* 71, 432-437, 2004.

Product for in vitro use only.

[cryotechlab@gmail.com](mailto:cryotechlab@gmail.com)